REVIEW

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Antiviral mechanisms targeting regulatory genes Tat and Rev to defeat latent HIV-1 infected T cells: a literature review

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ABSTRACT

Antiviral drug therapies have been utilized to prevent disease progression in patients positive HIV-1. Various research has been conducted to investigate and develop a potential functional therapy to suppress HIV-1 replication and cure latent HIV-1 in the absence of drugs. Approaches that have been well studied are the anti-HIV-1 which targets RNAs, proteins, or peptides expressed by HIV-1 resistant cells, which can be transplanted to the patients. RNA interference in the form of small RNA has been proven as a promising therapy to prevent HIV-1 replication. It is utilized for therapy using cell transplantation and various gene combinations in clinical trials. However, many studies have been failed to show a successful eradication of latently HIV-1 infected T cells. It is happened due to the virus's ability to escape from antiviral therapies. However, this can be overcome by using a combination of ARTs. On the other hand, genetic editing has been intensively studied since it can cure various diseases caused by genetic or pathogen infections, including HIV type 1. The previous studies have designed gRNA bind to protein Cas type 9 targeting HIV functional genes, Tat and Rev sequences. Various recombination has been introduced to CRISPR-based gene editing to increase the binding affinity and efficiency of Cas9 to target Tat and Rev proteins of their exons. The best approach for the Cas9 targeted Tat and Rev is by utilizing more than one guide RNA. However, Subsequent studies are needed to confirm the ability of Cas9 with various guide RNAs to inhibit virus activation and replication in latent HIV-1. This review aims to describe the mechanisms, advantages, and disadvantages of antiviral therapies that target Tat and Rev as regulatory genes to eradicate latent HIV-1 infected T cells.

Keywords: CRISPR, latent HIV-1, RNA- and protein-based therapies.
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INTRODUCTION

HIV-1 and HIV-2 have numerous similarities, including fundamental gene structure, transmission modes, intracellular replication pathways, and clinical outcomes: both outcomes in AIDS. HIV-1 is portrayed by higher transmissibility and high progression probability to AIDS. Epidemiologically, HIV-2 remaining mostly occurs in West Africa, while HIV-1 broadens around the world. Clinically, HIV-2 tainted people appear to dichotomize; most stay as long-term non-progressors, even though most people with HIV-1 infection progress. Plasma viral burdens are reliably lower in HIV-2 and the average magnitude of immune stimulation activation. Cellular responses to HIV-2 accomplished more polyfunctional and produce higher IL-2; humoral immune responses seem more extensive with lower extent intra type neutralization reactions. The immune response elicited by HIV-2 shows up more protective against infection progression proposing that critical insusceptible elements limit viral pathology. If such immune response can be replicated or instigated in people with HIV-1 infection, they may expand survival and decrease prerequisites for antiretroviral therapy.[1,2]

The significant medical development in managing people with HIV-1 infection has been the antiretroviral (ARV) drugs, including nucleoside-analog reverse transcriptase inhibitors (NRTIs), non–nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors, protease inhibitors (PIs), fusion inhibitors, and coreceptor antagonists. ARV combination drastically suppresses viral replication and decreases the plasma viral load of HIV-1 to underneath the constraints of recognizing the most sensitive clinical tests (<50 RNA copies/mL), bringing about a critical reconstitution of the immune system. Although ART ceases HIV replication by targeting various strides in the HIV life cycle, it could not dispose of the necromancy provirus consolidated into the host-cell genome. Latent proviruses have competency in replicating and able to bounce back in instances of ART interference or suspension. Generally, the small population regarding cells harbors provirus being a reservoir of HIV patients with ART-controlled and can express

small amounts to none of HIV RNA and HIV protein.[3,4]

Furthermore, beyond the resting memory CD4+ T cells, a reservoir of HIV additionally exists inside macrophages, myeloid cells, cerebrum microglial cells, gut epithelial cells, and hematopoietic stem cells (HSCs). The resting memory of CD4+ T cell has been the most widely studied and is considered the significant impediment to eradicating HIV-1. Notwithstanding an absence of dynamic viral production, patients with latent HIV-1 infection keep on demonstrating deviant cellular signaling and metabolic disorder, prompting minor to major systemic and cellular comorbidities and complications. These involve incorporate damage of genomic DNA; attrition of telomere; dysfunction of mitochondrial; premature aging; and lymphocytic, hepatic, renal, cardiovascular, and pneumonic dysfunctions.[5,6,7] This review highlights the benefits and limitations of RNA-, protein-based therapies as anti-HIV-1 infected T cells and promising CRISPR-based gene therapy to be further developed to target specific regulatory genes (Tat and Rev) in order to cure latent HIV-1.

Molecular features of HIV-1

HIV-1 genome is around 9.8 kb long includes two viral long-terminal repeats (LTRs) situated on both end points as incorporated into the host genome. The genome additionally includes gene encoding for structural protein (Gag, Pol, Env), regulatory proteins (Tat, Rev), and accessory proteins (Vpu, Vpr, Vif, Nef). Tat and Rev are essential genes, expression regulators. Tat does not just play a significant role in viral transcription and replication, but it can also actuate cellular gene assortment and be a neurotoxic protein. Tat and Rev have an important role in virus gene expression regulation, respectively, by interacting with RNA target components inside the leader sequence of 5' untranslated and gene envelope. The effective late transcription of HIV-1 after activating the virus exceptionally depends on regulatory proteins (Tat, Rev) initial expression. Tat starts the elongation phase of nascent viral mRNA from the incorporated provirus. At the same time, the nuclear transport of unspliced transcripts is under the regulation of Rev. Within HIV-1-activated T cells, Tat and Rev's proteins give an exceptionally significant degree of viral gene expression; meanwhile, similar proteins in idle T cells are essential for continuing the provirus in latent phase. Tat and Rev up are pondered as the most effectively and functionally conserved HIV-1 genes, with some domains of its genomic inside possess similar homology across extensive subtypes of HIV-1 and HIV-2.[2,8,9,10]

Vigorous reciprocity linking the activation of HIV-1 infected T cell and viral regulatory proteins, particularly Tat, is a significant regulator of HIV-1 latency state and reactivation. When CD4+ T cell goes through an effector-to-memory transition of T cell, it causes impermanent upregulation in the expression of CCR5, a viral coreceptor alongside CD4 works with HIV passage into the cell, and a rapid downregulation in the expression of gene expression. HIV in these cells is portrayed by repressed transcription of the HIV gene, which builds up latency. In a population study of recently infected primary T cells, an examination of viral RNA using single-cell RNAseq demonstrates that the transcriptional scheme of T cells by central memory marker and naïve T cells are related to viral dormancy. At the same time, Tat has been embroiled as controller master of HIV latency, nevertheless the cell states. That is fundamental for transcription and replication of the virus; accordingly, antiviral proxies that halt Tat capacity may obstruct virus production by infected cells. Presently, practical management for HIV-1 has been suggested to utilize a block-and-lock methodology, in which anti-HIV proxies that abort HIV-1 transcription lock the reservoir of cellular HIV to a deep-latent, transcriptionally silent state, block rebound following combined antiretroviral therapy (cART). Since Tat transactivation is a basic advance in HIV-1 transcription, blocking agents of Tat will hinder the viral replication and transcription.[8,9]

Numerous moieties of RNA-based and protein-based anti-HIV-1 targeting Tat and Rev proteins or the exons have been effectively demonstrated to lessen viral replication in T cells to a various level with techniques including, however, not being restricted to Tat/Rev up short hairpin RNA (shRNA), antisense RNA, a trans-activation response/Rev response element (TAR/RRE), mutant elements, Tat-/Rev- targeting on ribozymes. CRISPR/Cas9 framework abrogated Tat and Rev protein expression and their regulatory functions within steady 293 T cells expressing Tat-/Rev protein. Target-site sequencing affirmed that mutation associated with Cas9 occurs in Tat and Rev exons. Meanwhile, no off-target mutations were recognized in similar sequences with guide RNAs (gRNA) in the human genome. CRISPR transduction effectively decreased production of the viral capsid is a persistent and latent infection of CD4+ T-cell lines. These outcomes support the possible utilization of CRISPR to target HIV-1 regulatory genes specifically and conquer viral replication. Current modeling proposes inhibitors interface with Tat-/Rev protein and interrupt transactivation cycle, prompting epigenetic changes of nucleosomes in the viral promoter can delay extending inactivation, or deep dormancy, of HIV transcription.[11,12,13]

Tat/Rev short hairpin RNA (shRNA)

RNA interference (RNAi) has the ability to inhibit gene expression. Short RNA particles usually are made that are reciprocal to endogenous mRNA and, when brought into cells, tie to target mRNA. Target mRNA will be inactivated functionally by short RNA binding. Furthermore, it can cause target mRNA degradation. There have been two kinds of short RNA particles applied for RNAi. Small interfering RNA (siRNA) is a double-stranded RNA molecule, about 20-25 nucleotides in length. siRNA will refrain target mRNA until the degradation process inside the cell. shRNA is about 80 bps length include internal hybridization domain creating hairpin shape. siRNA is constructed from processed shRNA molecules. siRNA leads to the knockdown of gene expression. shRNA can be integrated into plasmid vectors and incorporated into genomic DNA, resulting in a longer knockdown of target mRNA because of its stable expression. Functional screening of RNAi knockdown required constructive shRNA design. In

constructing optimum sequence, there are multi factors affecting shRNA efficacy. Additionally, amplification and library manufacture issues arise by shRNA stem-loop hairpin arrangement.[11-17]

siRNAs and shRNAs used to virtually target Tat, Rev, Gag, Pol, Nef, Vif, Env, Env, Vpr, and LTR in HIV-encoded RNA inside cell lines. A previous study showed that lentiviral vectors induce anti-CCR5 and CXCR4 shRNAs combination inside human lymphocytes. Downregulation of the receptors leads to viral infectivity inhibition completely compare to controls. Nevertheless, as CXCR4 is fundamental for homing hematopoietic stem cells to marrow and differentiation of T cells, targeting CXCR4 is thought not the right option as anti-HIV therapy, nor targeting fundamental CD4 receptor. As a receptor of dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) is competent to be targeted by siRNAs to halt infection. Targeting only CCR5 coreceptors raises issues as HIV-1 shifts to CXCR4 in the AIDS course producing the abundant virulent infection. An effective and successful approach using RNAi should be able to cover those disadvantages. In case of those issues, incorporation of shRNAs into the Tat promoter can defeat fundamental cellular targets.[17-20]

Antisense RNA therapy

Present proofs suggest that the natural antisense of mammalian RNAs has significant roles for cellular homeostasis by regulating gene expression. Characterization and identification of retroviral antisense RNA (asRNA) will give novel apprehension regarding mechanisms of pathogenesis and replication. Previous studies have reported HIV-1 encoded asRNA and its essential role in viral infection. A study by Ishihara et al. demonstrated the depiction of HEK293T transcripts that were transferred transiently with an expressed plasmid of HIV-1_{NL4-3} DNA within the antisense alignment indicates numerous antisense transcripts credibly expressed. The main structure of an utmost HIV-1 antisense RNAs structure is related to a variation of former antisense protein (ASP) mRNA, which is 2.6 kb RNA that

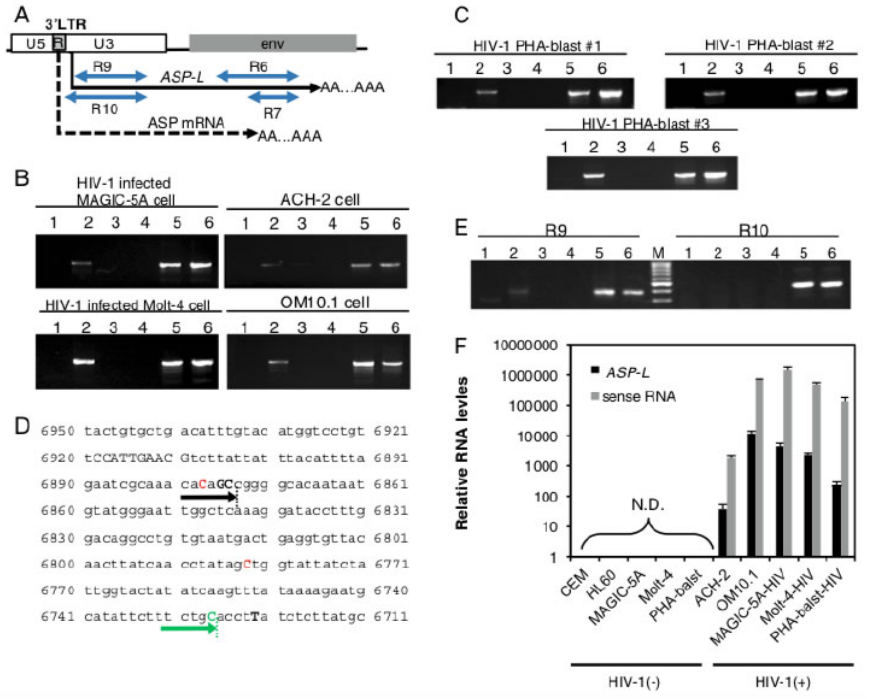

Figure 1. HIV-1 antisense RNA expression in numerous kinds of HIV-1 infected cells.[21]

transcribed through U3 domain of 3'3LTR and terminated on envelope area in acute or chronic cell lines infection and acute human peripheral blood mononuclear cells. The reporter probe exhibit HIV-1 LTR anchors promoter activity in reverse alignment. Analysis of mutation suggested implication of NF-κB binding sites in antisense transcription regulation. asRNA situated in nuclei of infected cells. Expression of antisense RNA able to suppress HIV-1 replication for >1 month. Moreover, the specific knockdown of asRNA magnifies replication and gene expression of HIV-1.[21]

RNA samples of Molt-4 infected acutely with HIV-1_{NL4-3} were analyzed to investigate HIV-1 expression asRNA in numerous HIV-1 infected cells. The investigation was also done in chronic infection of HIV-1_{IIIB} in cell lines of ACH-2 and OM10.1 by using specific antisense of RT-PCR. Figure 1A shows Michael et al. report of antisense-specific RT PCR by Tag-RT-primer with no amplification of ASP mRNA; Figure 1B depicts asRNA expression in all cell lines. Figure 1C exhibits detection of asRNA in PHA activated of peripheral blood mononuclear cells that infected by HIV-1_{NL4-3}. In addition, transcription stopping in OM10.1 and in ACH-2 activated cells, in which HIV-1_{IIB} has conserved polyadenylation signal, were revealed by 3' RACE examination (Figure 1D). Analysis of antisense-specific RT-PCR demonstrates TSS of ASP-L of HIV-1_{IIB} situated in between 9441 and 9538 nucleotide positions correlated with HIV_{NL4-3} (Figure 1E). The highest level of expression is in OM10.1, whereas HIV-1 asRNAs expression level was 100-2,500 lesser than ones in a sense RNA transcript inside all cells (Figure 1F).[21]

Downregulation of viral gene replication and expression was done by forced expression of ASP-L. Ishihara et al. subsequently examined ASP-L biologic effects in HIV-1 infected cells, then inaugurated Molt-4 transformants, which steadily express shRNA targeting HIV-1 asRNAs (shRNA#1 and shRNA#2 shown by Figure 2A). The possible intrusion at odds with ssRNAs, some mutated nucleotides were inaugurated to passenger strands targeting sense HIV-1 RNAs. Initially, shRNAs specificity was examined by luciferase reporters who possess a sense or antisense alignment in those transformants. Both shRNAs specifically diminished the luciferase

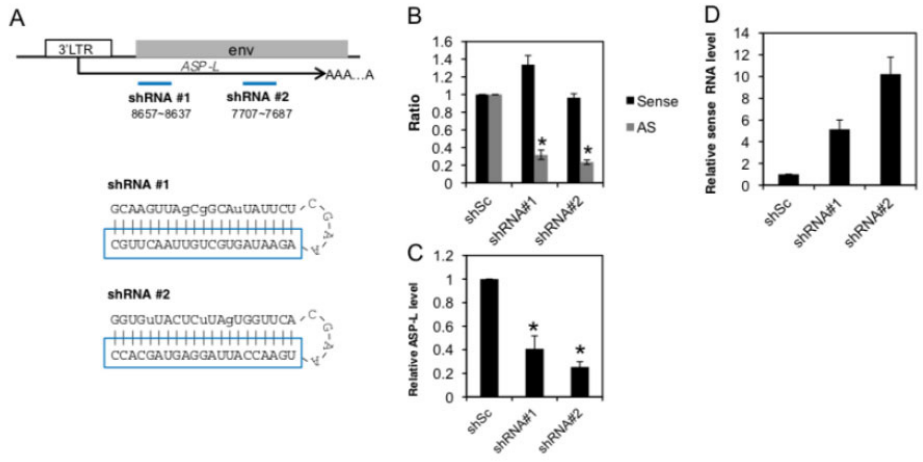

Figure 2. Endogenous HIV-1 antisense RNA effects on HIV-1 expression.[21]

pursuit of reporters possess ASP-L sequence; meanwhile, the outcomes were not significant for reporters possessing ssRNA (Figure 2B). Then, examination of shRNAs effects in HIV-1 replication. Figure 2C depicts that compared to control cells, HIV-1 asRNAs expression levels in the infected cell were suppressed. Significant intensification of sense HIV-1 RNAs sense levels in ASP-L-knockdown was demonstrated by qRT-PCR of RNA samples of infected cells compared to control cells (Figure 2D).[21]

TAR/RRE (Trans-activation response/ Rev response element) decoy

Decoy RNA/DNA has been developed as one of the antiviral strategies, including for HIV-1 latency. Due to their stable shapes, these short RNA/DNA chains or aptamers bind to their target with high affinity.[21] Besides, rather than interact with viral DNA/RNA, these small aptamers act specifically to bind to a viral target protein which has a crucial task in the viral replication process. The binding between the decoy and target protein prevents the virus replication by inactivating those proteins.[22] Most aptamers have a three-dimensional structure like hairpin monomers; therefore, it is possible to have duplexes[23], triplexes[24], or even quadruplexes[25,26] function. However, it is not many aptamers have been known their particular applications. Thus, the systematic evolution of ligands by exponential enrichment (SELEX) has been utilized to select 20-100 unique sequences with a high binding affinity to a certain protein family to inhibit viral replication and function.[27] Aptamers are producible and economically synthesized on a large scale for medical applications. As well as other RNA therapeutics, RNA aptamers are often modified during chemical synthesis to reduce the activation of nucleases and improve their pathological function. The chemical modification includes 20 -F, 20 -OMe, sugar substitutions in LNA, or forming spiegelmer aptamer. In addition, cholesterol or polyethylene glycol (PEG) can be conjugated to the aptamers to reduce antibody response and renal filtration.[27]

HIV-1 formed DNA using their reverse transcriptase and integrated their DNA into host DNA. Utilizing the host's transcriptase system, the mRNA host contains mRNA HIV-1. It is important to ensure the mRNA virus does not undergo a splicing process before transferred to the cytosol host and translated to HIV-1' structural proteins.[28] Therefore, it is critical for the Rev response element (RRE) to bind with Rev proteins.[29] mRNA viruses contain RRE, which bind with Rev proteins in the nucleus, and this complex is exported to the cytoplasm without splicing.[30]

On the other hand, evading splicing is not the only crucial step in the HIV-1 replication process; initiating the transcription also plays an important role in HIV-1 replication. This process will be started when the Tat protein binds to Trans-activation responses (TAR) located at RNA complements. This complex then induces binding between promotor and transcription factors, activating the RNA polymerase II and the transcription elongation process. Therefore, besides RRE binding, TAR binding also can be a target for antiretroviral drugs.[31–33]

Mimicking the structure of transactivation response (TAR) and Rev response element (RRE) in HIV-1 with RNA decoy is a novel strategy to inhibit the HIV-1 regulatory proteins Tat and Rev.[34] Both RRE and TAR decoys have hairpin loop structures that specifically bind to Rev and Tat proteins. Additionally, TAR and RRE decoys have a higher affinity to bind with Tat or Rev proteins, which is why this method has the successful potential in treating latent HIV infection. However. During clinical trials for TAR and RRE decoy, it has been found that they do not affect the host cell cycle and its function. However, these treatments do not significantly reduce the HIV-1 level in plasma subjects.[28,35] Therefore, this treatment is given in combination with another antiretroviral treatment mechanism.

Tat/Rev-targeting ribozyme

Ribozyme is an RNA that cleavage, ligation, and peptide form enzymes which self-processing, meaning do not require catalysis proteins. Besides, it was found that ribozyme can specifically recognize certain sequences then cleavage them. The recognition domain of ribozyme can be modified to recognize a specific sequence, which leads to site-specific cleavage.[36] Moreover, as an engineering development process, ribozymes undergo several selections using in vitro tests and direct evolution tests to improve their chemical and biological properties as therapeutical agents. As chemical engineering modification, ribozymes were engineered to form an allosteric molecule activated by effector molecules, called ''riboswitches".[37,38] There are several types of ribozymes based on their specific functions. However, hammerhead and hairpin ribozymes have been studied intensively because both have great potential as antiviral therapy. In terms of the delivery process, ribozyme therapeutic can either be delivered as RNA or as a therapeutic gene. Due to the lack of stability, if ribozyme will be delivered

as fully-RNA form, it requires stabilizer modification, including terminal inverted 30 -30 deoxy abasic nucleotides, 50 -PS backbone linkage, 20 -O-Me, and 20 -deoxy-20 -C-allyl uridine.[39]

Various ribozymes have been developed and clinically tested against HIV-1 as a gene therapy for infected hematopoietic stem cells and T cells positive HIV-1.[40–42] Since HIV-1 mostly infects CD4+ T cells, it is crucial to develop a therapeutic system that will inhibit infected CD4+ T (latent T cell) producing functional HIV-1 virus. The in vitro or ex vivo trials utilized either T cells from the patient (autologous) or T cells from an identical twin (syngeneic), which, after the cells are harvested, then is treated with the ribozyme-embedded gene therapy and reinfused back into the patient. In vivo test has been done by utilizing Retroviral vectors, facilitating the integration of the therapeutic genes into the host genome, and showing long-term gene expression after integration.[29,43]

Intensive research has been done on the effect of synthetic ribozymes to target gene Tat and Rev in HIV-1. after the mRNA containing Tat and Rev is transported to the cytoplasm host, synthetic ribozymes will bind to Tat and Rev mRNA, then catalyze the cleavage of those genes. Synthetic hammerhead ribozymes were studied extensively and showed that they successfully cleaved Tat and Rev RNA or exon for Tat and Rev. While all those technologies have shown high feasibility to produce and high safety level for the host, none of the engineered ribozymes demonstrated a limited efficacy to reduce HIV-1 production from infected CD4+ T cells.[44]

CRISPR-based gene editing

Gene editing-based therapy has been a novel innovation for many diseases, like cancer and other infectious diseases, which still be a complicated matter to cure. zinc finger nuclease (ZFN) and transcription activator-like effector nuclease (TALEN) are site-directed engineered nucleases based on DNA-protein recognition mechanism. They lead FokI nuclease directly to any specific sequence within the genome target and digest it. Regardless of the remarkable finding, difficulties are faced in the synthesis process since this technology is specifically made for each type of gene interest.[45] The fantastic finding of bacterial immune system mechanism in which potential for gene editing enlargement is known as CRISPR (the clustered regularly interspaced short palindromic repeats). RNA molecule called guide-RNA (gRNA) in CRISPR-associated 9 (Cas9) system is able to guide this complex to a specific sequence target. As a type of nuclease protein, Cas9 has the ability to initiate double-strand breaks (DSB) within DNA with highly efficient and specific. Besides, to utilize Cas9 for difference sequence interset, it can easily be received by modifying gRNA sequence.[46] As the newest system developed in gene editing, CRISPR outperform ZFN and TALEN since it is less complex to package into a lentiviral vector as cloning and delivery system, rarely perform off-target cleavage[45,47] less costly as it is easier to create, and showing high efficacy and promising results on infectious and genetic diseases[48] including HIV-1.[49]

Several studies have proven that CRISPR cleavages within HIV-1 LTR by inducing double-strand breaks at 5' end and 3' end.[50] Several mechanisms and Cas9 targets have been studied to find the most efficient mechanism to prevent HIV-1 replication within the cells using CRISPR-based. Subsequent studies failed to show any excision in structural or functional protein genes in the HIV-1 genome. The host's DNA repair mechanism presumably causes it. The other study, which targets RRE to suppress nuclear export of HIV-1 genome[48,49], shows that bases chain variation in RRE can affect the efficiency of gRNA and does not have a significant effect on inhibiting Rev and RRE binding.[51] Therefore, targeting Rev gene transcription or translation process will be more suitable to suppress HIV-1 replication. Daugherty et al. demonstrated that gRNA targeted Rev subdomain B and Tat subdomain A, *in vitro* and *ex vivo* studies, achieve the best result to suppress HIV-1 in infected T cells. RevB and TatA sequences are predicted as a targeted motif for protein function; therefore, by using both as Cas9 targets, the highest replication inhibition can be received.[52] However, mutation characteristics and efficiency of CRISPR may eventually have significant roles in increasing the inhibition capacity. It is still undefined whether the high frequency of indels mutations or frameshift mutations are the main factors responsible for the wide range of efficiency binding to a gene of interest between different gRNAs.[53]

Latent HIV-1 is in the inactive phase, and since in this state, the virus lacks reverse transcriptase factors, indicates minor mutations associated with resistance have occurred. However, in this state, HIV-1 still competent to escape from antiretroviral single therapy and ZFN.[54] Although only has a minor mutation, mutations that happened at the PAM recognition site have the possibility of causing resistance to subsequence Cas9 proteins activities.[55,56] However, recent studies demonstrated that utilizing more than one gRNA targeting the natural and possible mutant sequences causes multiple Cas9 cleavage and saturation of virus gene sequences, therefore preventing virus escape. This mechanism is an analogue to the ART combination strategy.[53] Ophinni et al. mentioned that the most efficient combination to prevent latent HIV-1 replication is by using multiplex gRNAs consist of six gRNAs targeted Tat and Rev.[57] However, subsequent in vivo and preclinical studies need to carry out to make sure there is no negative impact on the host and no off-target cleavage due to using several gRNAs.

Even though CRISPR conveys various benefits to improve gene editing-based therapy for curing HIV infection, it still has a numerous weakness which needs to overcome. CRISPR efficiency and specificity depend on guide RNA. One of the greatest obstacles in CRISPR technology is the off-target mechanism caused by mismatched pairing between guide RNA and DNA target.[58] Mismatches can be happened due to mutation; therefore, gRNA cannot identify the DNA target.[59] On the other hand, off-target can also cause random cleavage to the DNA non-target, leading to mutation such as deletion, insertion, and point mutation that knockdown gene function.[60] Accordingly, numerous studies have proved that the CRISPR activity varies depending on the levels of mismatches between gRNA and target DNA. Most of the previous

publications show that CRISPR tolerated single and double mismatches of gRNA[61-65], however, more mismatches of gRNA reduce, moreover obliterate the nuclease activity. Another research demonstrated that five or more mismatches located distant to the target sequence also lead to off-target. It proves other factors that also influence the possibility of off-target happened, such as GC content within DNA target.[63]

Several modifications targeted Cas9 and gRNA are developed and studied to improve the efficiency of CRISPR-CAs9 activity, including introduce several point mutations on the Cas9[66], utilizing less than 20 nucleotides for gRNA sequence (truncated gRNA)[67], and add additional hairpin structures on the gRNA[68,69]. These methods successfully reduce the off-target of CRISPR-CAs9. However, further research is necessitated to prove the stability of CRISPR-CAs9 activity with those modifications to different DNA targets.

CONCLUSION

Early expression of functional proteins Tat and Rev have an important contribution to successfully regulate replication and followed by HIV-1 activation. Tat initiates the transcription from the integrated provirus. At the same time, the nuclear export of unspliced transcripts is regulated by Rev. Tat and Rev are highly conserved genes of HIV-1 since both genes have similar sequences within HIV-1 subtypes. Many anti-HIV-1 therapies targeting Tat and Rev proteins or their genes have been successfully suppressed viral replication in T using various mechanisms, including RNA interferences (example: antisense and short hairpin RNA), TAR, and RRE decoys, mutant molecules, and engineered ribozyme. CRISPR/Cas9 shows as an encouraging method to target Tat and Rev genes. However, antiretroviral monotherapy significantly reduces viral levels when the latent HIV-1 infected T cells are activated. Although, that therapy specifically targets the Rev and Tat proteins or their gene. Therefore, previous studies suggest performing RNA-based and protein-based combination antiviral therapies. As a final goal to cure latent HIV-1, various techniques have been studied, including gene editing, like ZFN and TALEN. This method denotes a high potential to cure various diseases, including eradicating latent HIV-1. However, advanced technology and high cost are needed to develop this therapy. Fortunately, a novel breakthrough finding regarding gene editing development has been found and successfully synthesized. CRISPR-based gene editing does not advance technology and cost-effective to produce. Additionally, Cas9 can be specifically targeted gene Tat and Rev. However, although the mutation rarely found at that gene sequence due to lack of polymerization in latent HIV-1, some mutations still happened and lead to resistance to some types of CRISPRs. This obstacle is successfully overcome by utilizing more than one guide RNA as a part of the CRISPR system. Subsequent studies are required to confirm that CRISPR-based gene therapy is a safe treatment for curing latent HIV-1.

DISCLOSURES

Conflicts of interest

There have been no competing interests regarding this manuscript.

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Ethical Statement

Not applicable.

Author contribution

The first author is the guarantor and constructs the concept of the manuscript. Design, intelligent content description, literature quest, data collection, data processing, manuscript writing, manuscript editing, and manuscript review are contributed by all authors.

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